

DESCRIPTION

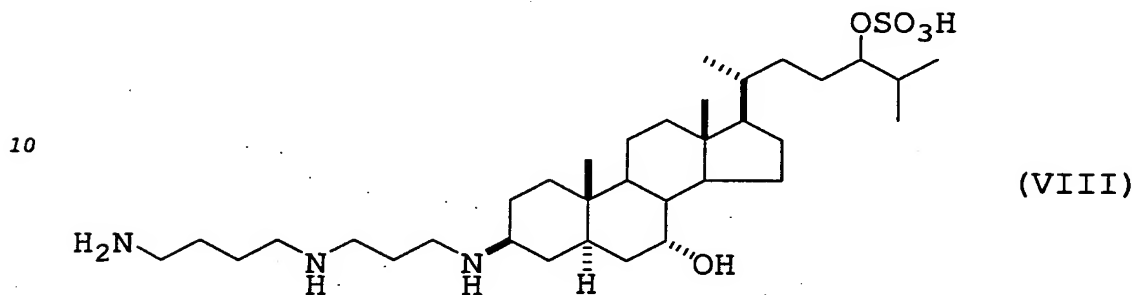
METHOD FOR PRODUCING 5 α -PREGNANE DERIVATIVE

Technical Field

The present invention relates to production methods of
 5 5 α -pregnane derivatives useful as synthetic intermediates for
 squalamine.

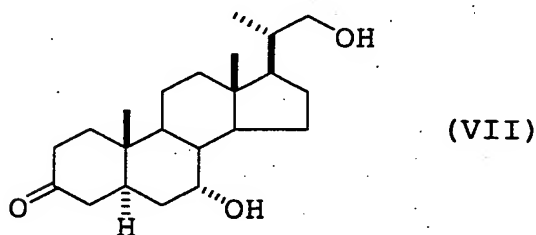
Background Art

Squalamine is a compound represented by the formula
 (VIII):

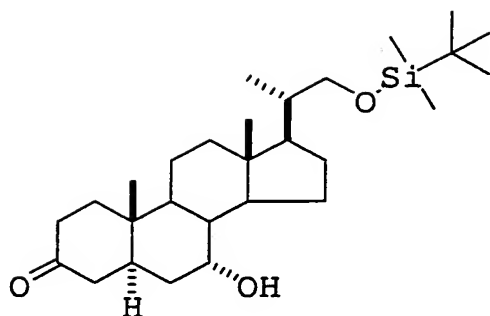


, which has been reported to show strong antibacterial
 activity against Gram-positive bacteria, Gram-negative
 bacteria, fungi and the like, as well as anticancer activity,
 and is drawing attention as a new antibiotic.

15 Conventionally, squalamine is extracted from the liver of
 dogfish. In view of its extremely low content of 0.001-0.002
 wt% and poor extraction efficiency, however, various chemical
 synthetic methods have been studied. Particularly, (20S)-
 7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one represented by the
 20 formula (VII):



(W001/79255, Organic Letters, Vol. 2, p. 2921 (2000)) and
 (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -
 pregn-3-one represented by the formula (IX):



(IX)

(W003/51904) are known to be useful intermediates that can be converted to squalamine comparatively in a few steps.

Conventionally, as production methods of (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one, a method (W001/79255) including subjecting (20S)-7 α ,21-dihydroxy-20-methylpregn-4-en-3-one to what is called the Birch reduction using not less than 30 equivalents of metal lithium in liquid ammonia with the aim of stereoselective reduction to an α form in the 5-position, a method (W002/20552) including subjecting (20S)-7 α ,21-dihydroxy-20-methylpregna-1,4-dien-3-one to the Birch reduction using 10 equivalents of metal lithium in liquid ammonia, and the like have been developed.

In addition, as a production method of (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-3-one, a method (W003/51904) including reducing (20S)-7 α ,21-dihydroxy-20-methylpregna-1,4-dien-3-one in the same manner as in the above to give (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one, and then protecting the hydroxyl group at the 21-position of the compound with a tert-butyldimethylsilyl group is known.

Disclosure of the Invention

However, the yield of the above-mentioned method is 71% at the highest and, in consideration of the fact that the pregnane derivative is an expensive raw material, the method cannot be considered a preferable production method but has a room for improvement before its industrial practice.

In the aforementioned conventional reaction, a ketone derivative stereoselectively converted to a 5 α form is

obtained from an unsaturated ketone having at least the carbon-carbon double bond at 4- and 5-positions as a raw compound, which corresponds to what is called the partial reduction where an unsaturated ketone is converted to a saturated ketone. According to the conventional reaction, however, it has been clarified that the saturated ketone is further reduced to give an alcohol form due to the side reaction. To suppress such side reaction, it is important that the reaction be carried out using a reducing agent in an amount close to the equivalent amount, which is necessary for the partial reduction. However, in the conventional reaction, a large excess of metal lithium is used.

The present inventors have found that the conventional methods are low in the yield because a hydroxyl group is present at the 21-position of the raw material pregnane derivative, namely, since metal lithium, which is a reducing agent, is decomposed due to the highly reactive primary hydroxyl group present at the 21-position to lose reducing ability, use of excess metal lithium is unavoidable, and that since the primary hydroxyl group also acts as a proton donor during the reduction reaction, which in turn further promotes the reaction, an alcohol form is by-produced.

Based on such finding, the present inventors have found that, by a reduction reaction using a compound wherein the hydroxyl group at the 21-position is protected as a raw compound, the decomposition of the reducing agent due to the hydroxyl group and the action as a proton donor are suppressed, the amount of a reducing agent to be used can be markedly reduced as compared to conventional methods, reduction of saturated ketone is significantly suppressed, and the objective 5 α -saturated ketone can be obtained in a high yield. On the other hand, however, it has been clarified as a problem that a 5 α -1-en-3-one form having an extremely similar chemical structure to that of the objective 5 α -saturated

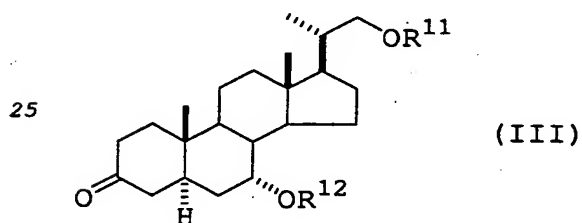
ketone remains as an impurity and its separation and removal is extremely difficult by conventional methods such as recrystallization, column chromatography and the like. This is deeply problematic as a production method of an intermediate
5 for pharmaceutical products generally required to have high purity.

Therefore, an object of the present invention is to provide a method of producing a (20S)-7 α ,21-dihydroxy-20-methylpregna-3-one derivative, which is useful as an
10 intermediate for squalamine, which method obliterates removal of a 5 α -1-en-3-one form which is an impurity difficult to be removed by purification.

The present inventors have found that, by subjecting a mixture of the objective 5 α -saturated ketone form and a 5 α -1-en-3-one form, which is an impurity, to a reduction reaction,
15 only a carbon-carbon double bond of the 5 α -1-en-3-one form is reduced without impairing the 5 α -saturated ketone form in the mixture, and therefore, the objective 5 α -saturated ketone form is converted, which resulted in the completion of the present
20 invention.

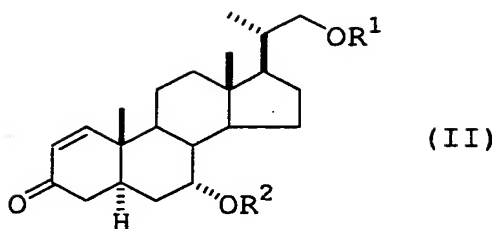
Accordingly, the present invention provides the following.

(1) A method for producing a 5 α -pregnane derivative represented by the formula (III):

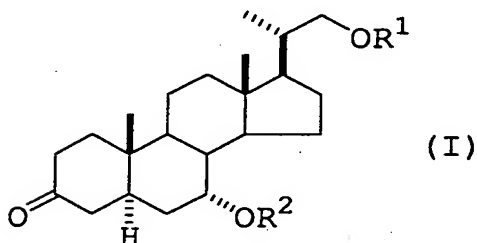


wherein R¹¹ and R¹² are each independently a hydrogen atom or a hydroxyl-protecting group (hereinafter sometimes to be referred to as compound (III) in the present specification), which comprises selectively reducing a carbon-carbon double
30 bond of a 5 α -pregnane derivative represented by the formula

(II):



wherein R^1 and R^2 are each independently a hydrogen atom or a hydroxyl-protecting group (hereinafter sometimes to be referred to as compound (II) in the present specification), in a mixture of a 5α -pregnane derivative represented by the formula (I):



wherein R^1 and R^2 are as defined above (hereinafter sometimes to be referred to as compound (I) in the present specification), and compound (II).

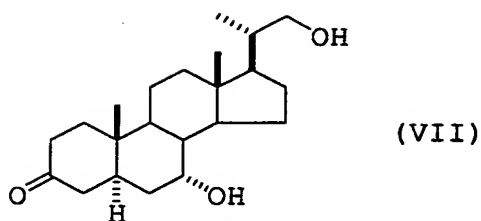
(2) The method of the above-mentioned (1), wherein R^2 and R^{12} are hydrogen atoms.

(3) The method of the above-mentioned (2), wherein R^1 and R^{11} are tri-substituted silyl groups having three, same or different, substituents selected from the group consisting of an alkyl group optionally having substituent(s), an aryl group optionally having substituent(s), an alkoxyl group optionally having substituent(s) and an aryloxy group optionally having substituent(s).

(4) The method of the above-mentioned (3), wherein R^1 and R^{11} are tert-butyldimethylsilyl groups.

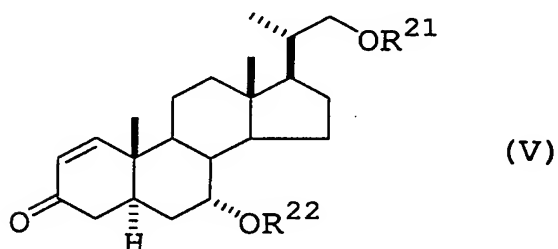
(5) The method of the above-mentioned (2), wherein R^1 and R^{11} are hydrogen atoms.

(6) A method for producing (20S)- $7\alpha,21$ -dihydroxy-20-methyl- 5α -pregn-3-one represented by the formula (VII):

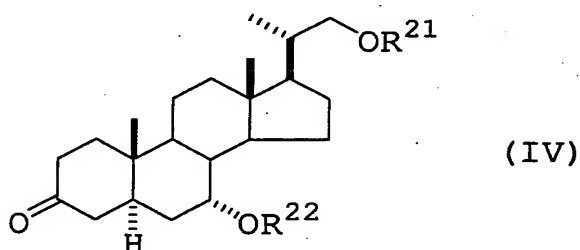


(hereinafter sometimes to be referred to as compound (VII) in the present specification), which comprises

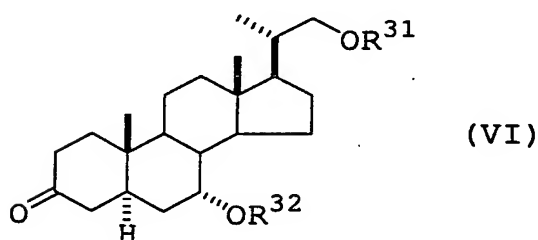
(a) selectively reducing a carbon-carbon double bond of a 5α-
 5 pregnane derivative represented by the formula (V):



wherein R²¹ is a hydroxyl-protecting group and R²² is a hydrogen
 atom or a hydroxyl-protecting group (hereinafter sometimes to
 be referred to as compound (V) in the present specification),
 10 in a mixture of a 5α-pregnane derivative represented by the
 formula (IV):



wherein R²¹ and R²² are as defined above (hereinafter sometimes
 to be referred to as compound (IV) in the present
 15 specification), and compound (V) to give a 5α-pregnane
 derivative represented by the formula (VI):



wherein R^{31} is a hydroxyl-protecting group and R^{32} is a hydrogen atom or a hydroxyl-protecting group (hereinafter sometimes to be referred to as compound (VI) in the present specification); and

5 (b) eliminating the hydroxyl-protecting groups of compound (VI) obtained by the aforementioned step.

(7) The method of the above-mentioned (6), wherein R^{22} and R^{32} are hydrogen atoms.

(8) The method of the above-mentioned (7), wherein R^{21} and R^{31}
10 are tri-substituted silyl groups defined above.

(9) The method of the above-mentioned (8), wherein R^{21} and R^{31} are tert-butyldimethylsilyl groups.

According to the method of the present invention, by
subjecting a (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-1-en-3-
15 one derivative contained in a (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one derivative (5 α -saturated ketone form) and
difficult to be separated to a reduction reaction in the form
of the mixture, only a carbon-carbon double bond of the 5 α -1-
en-3-one form is reduced to convert the derivative to the
20 objective 5 α -saturated ketone form to be effectively used,
without impairing the 5 α -saturated ketone form which is the
objective product in the mixture. According to the present
invention, moreover, by using a compound wherein the hydroxyl
group at the 21-position is protected as a raw compound for
25 producing a 5 α -pregnane derivative by stereoselectively
reducing a pregna-1,4-diene derivative, the amount of the
reducing agent can be decreased, a side production of an
alcohol form due to promoted reaction can be suppressed,
whereby a 5 α -pregnane derivative useful as a synthetic
30 intermediate for squalamine can be produced in a high yield.

Best Mode for Embodying the Invention

1. Explanation of symbols

In the above-mentioned formulas, the hydroxyl-protecting group represented by R^1 , R^2 , R^{11} , R^{12} , R^{21} , R^{22} , R^{31} or R^{32} may be

any as long as it acts as a hydroxyl-protecting group and, for example, an alkyl group optionally having substituent(s); an acyl group optionally having substituent(s) (e.g., a formyl group, an alkylcarbonyl group optionally having
5 substituent(s), an alkenylcarbonyl group optionally having substituent(s), an arylcarbonyl group optionally having substituent(s) etc.); an alkoxycarbonyl group optionally having substituent(s); an aryloxy carbonyl group optionally having substituent(s); a carbamoyl group (e.g., a carbamoyl
10 group wherein the nitrogen atom is optionally substituted by an alkyl group optionally having substituent(s) or an aryl group optionally having substituent(s)); a tri-substituted silyl group (the tri-substituted silyl group has three, same or different, substituents selected from the group consisting
15 of an alkyl group optionally having substituent(s), an aryl group optionally having substituent(s), an alkoxyl group optionally having substituent(s) and an aryloxy group optionally having substituent(s)); and the like can be mentioned.

20 The alkyl group as the hydroxyl-protecting group represented by R^1 , R^2 , R^{11} , R^{12} , R^{21} , R^{22} , R^{31} or R^{32} ; the alkyl group as a part of the acyl group and the alkyl group as a substituent that the acyl group optionally has; the alkyl group as a part of the alkoxycarbonyl group; the alkyl group
25 as a substituent that the carbamoyl group optionally has; the alkyl group that the tri-substituted silyl group has, the alkyl group as a part of the alkoxyl group that the tri-substituted silyl group has, and the alkyl group as a substituent that the aryl group and aryloxy group that the
30 tri-substituted silyl group has optionally have, may be linear, branched or cyclic, and preferably has 1 to 12, more preferably 1 to 8, carbon atoms. As such alkyl group, for example, methyl group, ethyl group, propyl group, isopropyl group, butyl group, isobutyl group, tert-butyl group, hexyl

group, octyl group, dodecyl group, cyclopentyl group, cyclohexyl group and the like can be mentioned.

The above-mentioned alkyl group optionally has substituent(s). While the number of substituents is not particularly limited, 1 to 6 is preferable, and when the number is two or more, the substituents may be the same or different. As such substituent, for example, an aryl group having 6 to 12, preferably 6 to 10, carbon atoms, which optionally has substituent(s), such as phenyl group, tolyl group, methoxyphenyl group, nitrophenyl group, naphthyl group, fluorenyl group and the like; an alkenyl group having 2 to 12, preferably 2 to 10, carbon atoms such as vinyl group and the like, which optionally has substituent(s); a linear, branched or cyclic alkoxyl group having 1 to 12, preferably 1 to 8, carbon atoms (the alkoxyl group may form a ring structure (e.g., tetrahydropyran ring, tetrahydrofuran ring etc.) together with an alkyl group which is a hydroxyl-protecting group) such as methoxy group, ethoxy group, propoxy group, isopropoxy group, butoxy group, isobutoxy group, tert-butoxy group, hexyloxy group, octyloxy group, dodecyloxy group, cyclopentyloxy group, cyclohexyloxy group and the like; an aralkyloxy group having 7 to 12, preferably 7 to 11, carbon atoms such as benzyloxy group and the like; an alkenyloxy group having 2 to 12, preferably 2 to 8, carbon atoms such as allyloxy group and the like; an aryloxy group having 6 to 12, preferably 6 to 10, carbon atoms such as phenoxy group, naphthyloxy group and the like, which optionally has substituent(s); and the like can be mentioned.

The alkenyl group as a part of the acyl group as the hydroxyl-protecting group represented by R^1 , R^2 , R^{11} , R^{12} , R^{21} , R^{22} , R^{31} or R^{32} , and the alkenyl group as a substituent that the acyl group optionally has; the alkenyl group as a substituent that the aryloxycarbonyl group optionally has; and the alkenyl group as a substituent that the aryl group, alkoxyl group and

aryloxy group that the tri-substituted silyl group has optionally have, may be linear, branched or cyclic, and preferably has 2 to 12, more preferably 2 to 8, carbon atoms. As such alkenyl group, for example, vinyl group, 1-methylvinyl group, 1-propenyl group, 1-octenyl group, 1-dodecenyl group, 1-cyclopentenyl group, 1-cyclohexenyl group and the like can be mentioned.

The above-mentioned alkenyl group optionally has substituent(s). While the number of substituents is not particularly limited, 1 to 6 is preferable, and when the number is two or more, the substituents may be the same or different. As such substituent, for example, an aryl group having 6 to 12, preferably 6 to 10, carbon atoms, which optionally has substituent(s), such as phenyl group, tolyl group, methoxyphenyl group, nitrophenyl group, naphthyl group, fluorenyl group and the like; a linear, branched or cyclic alkoxy group having 1 to 12, preferably 1 to 8, carbon atoms such as methoxy group, ethoxy group, propoxy group, isopropoxy group, butoxy group, isobutoxy group, tert-butoxy group, hexyloxy group, octyloxy group, dodecyloxy group, cyclopentyloxy group, cyclohexyloxy group and the like; an aralkyloxy group having 7 to 12, preferably 7 to 11, carbon atoms such as benzyloxy group and the like; an alkenyloxy group having 2 to 12, preferably 2 to 8, carbon atoms such as allyloxy group and the like; an aryloxy group having 6 to 12, preferably 6 to 10, carbon atoms such as phenoxy group, naphthyloxy group and the like, which optionally has substituent(s); and the like can be mentioned.

The aryl group as a part of the acyl group as the hydroxyl-protecting group represented by R^1 , R^2 , R^{11} , R^{12} , R^{21} , R^{22} , R^{31} or R^{32} , and the aryl group as a substituent that the acyl group optionally has; the aryl group as a part of the aryloxy carbonyl group and the aryl group as a substituent that the aryloxy carbonyl group optionally has; the aryl group as a

substituent that the carbamoyl group optionally has; the aryl group that the tri-substituted silyl group has, the aryl group as a part of the aryloxy group that the tri-substituted silyl group has, and the aryl group as a substituent that the aryl group, alkoxyl group and aryloxy group that the tri-substituted silyl group has optionally have, preferably have 6 to 10 carbon atoms and, for example, phenyl group, naphthyl group and the like can be mentioned.

The above-mentioned aryl group optionally has substituent(s). While the number of substituents is not particularly limited, 1 to 6 is preferable, and when the number is two or more, the substituents may be the same or different. As such substituent, for example, a linear, branched or cyclic alkyl group having 1 to 12, preferably 1 to 8, carbon atoms such as methyl group, ethyl group, propyl group, isopropyl group, butyl group, isobutyl group, tert-butyl group, hexyl group, octyl group, dodecyl group, cyclopentyl group, cyclohexyl group and the like; a linear, branched or cyclic alkoxyl group having 1 to 12, preferably 1 to 8, carbon atoms such as methoxy group, ethoxy group, propoxy group, isopropoxy group, butoxy group, isobutoxy group, tert-butoxy group, hexyloxy group, octyloxy group, dodecyloxy group, cyclopentyloxy group, cyclohexyloxy group and the like; a linear, branched or cyclic acyloxy group having 1 to 12, preferably 1 to 8, carbon atoms such as formyloxy group, acetyloxy group, propionyloxy group, butyryloxy group, isobutyryloxy group, valeryloxy group, isovaleryloxy group, pivaloyloxy group, hexanoyloxy group, octanoyloxy group, dodecanoyloxy group, cyclopentanecarbonyloxy group, cyclohexanecarbonyloxy group, benzoyloxy group, methoxybenzoyloxy group, nitrobenzoyloxy group and the like; nitro group; cyano group and the like can be mentioned.

Of the hydroxyl-protecting groups represented by R^1 , R^2 ,

R^{11} , R^{12} , R^{21} , R^{22} , R^{31} or R^{32} , specific examples of the alkyl group optionally having substituent(s) include methyl group, ethyl group, tert-butyl group, methoxymethyl group, tert-butoxymethyl group, benzyloxymethyl group, 2-tetrahydropyranyl group, 2-tetrahydrofuranyl group, 1-ethoxyethyl group, 1-benzyloxyethyl group, benzyl group, p-methoxybenzyl group, p-nitrobenzyl group, trityl group and the like, with preference given to methyl group, ethyl group, methoxymethyl group, 2-tetrahydropyranyl group, 2-tetrahydrofuranyl group and 1-ethoxyethyl group.

Of the hydroxyl-protecting groups represented by R^1 , R^2 , R^{11} , R^{12} , R^{21} , R^{22} , R^{31} or R^{32} , specific examples of the acyl group include formyl group, acetyl group, propionyl group, butyryl group, isobutyryl group, valeryl group, isovaleryl group, pivaloyl group, hexanoyl group, octanoyl group, dodecanoyl group, cyclopentanecarbonyl group, cyclohexanecarbonyl group, methoxyacetyl group, crotonoyl group, cinnamoyl group, phenylacetyl group, phenoxyacetyl group, benzoyl group, methoxybenzoyl group, nitrobenzoyl group and the like, with preference given to formyl group, acetyl group, propionyl group and pivaloyl group.

Of the hydroxyl-protecting groups represented by R^1 , R^2 , R^{11} , R^{12} , R^{21} , R^{22} , R^{31} or R^{32} , specific examples of the alkoxycarbonyl group optionally having substituent(s) include methoxycarbonyl group, ethoxycarbonyl group, propoxycarbonyl group, isopropoxycarbonyl group, butoxycarbonyl group, isobutoxycarbonyl group, tert-butoxycarbonyl group, hexyloxycarbonyl group, octyloxycarbonyl group, dodecyloxycarbonyl group, cyclopentyloxycarbonyl group, cyclohexyloxycarbonyl group, benzyloxycarbonyl group, p-methoxybenzyloxycarbonyl group, fluorenylmethoxycarbonyl group, p-nitrobenzyloxycarbonyl group, allyloxycarbonyl group and the like, with preference given to methoxycarbonyl group, ethoxycarbonyl group, isobutoxycarbonyl group and

allyloxycarbonyl group.

Of the hydroxyl-protecting groups represented by R^1 , R^2 , R^{11} , R^{12} , R^{21} , R^{22} , R^{31} or R^{32} , specific examples of the aryloxy carbonyl group optionally having substituent(s) include
5 phenoxy carbonyl group, p-nitrophenoxy carbonyl group and the like, with preference given to phenoxy carbonyl group.

Of the hydroxyl-protecting groups represented by R^1 , R^2 , R^{11} , R^{12} , R^{21} , R^{22} , R^{31} or R^{32} , specific examples of the carbamoyl group include carbamoyl group wherein any hydrogen atom that a
10 nitrogen atom has is optionally substituted, for example, by a linear, branched or cyclic alkyl group having 1 to 12 carbon atoms such as methyl group, ethyl group, propyl group, isopropyl group, butyl group, isobutyl group, tert-butyl group, hexyl group, octyl group, dodecyl group, cyclopentyl
15 group, cyclohexyl group and the like, an aralkyl group having 7 to 12 carbon atoms such as benzyl group and the like, an alkenyl group having 2 to 12 carbon atoms such as allyl group and the like or an aryl group having 6 to 10 carbon atoms, which optionally has substituent(s), such as phenyl group,
20 methoxyphenyl group, naphthyl group and the like, and the like.

Of the hydroxyl-protecting groups represented by R^1 , R^2 , R^{11} , R^{12} , R^{21} , R^{22} , R^{31} or R^{32} , specific examples of the tri-substituted silyl group include trimethylsilyl group,
25 triethylsilyl group, triisopropylsilyl group, dimethylisopropylsilyl group, diethylisopropylsilyl group, tert-butyldimethylsilyl group, tert-butyldiphenylsilyl group, tribenzylsilyl group, tert-butylmethoxyphenylsilyl group and the like, with preference given to tert-butyldimethylsilyl
30 group, triethylsilyl group and triisopropylsilyl group, and more preference given to tert-butyldimethylsilyl group.

As R^1 , R^{11} , R^{21} or R^{31} , a tri-substituted silyl group is preferable, and a tert-butyldimethylsilyl group is more preferable.

Since the hydroxyl group at the 7-position in a mixture of compound (I) and compound (II) or a mixture of compound (IV) and compound (V) may or may not be protected. However, since introductory reaction of the protecting group can be
5 omitted, it is preferably not protected. Therefore, as R^2 , R^{12} , R^{22} and R^{32} , hydrogen atoms are preferable.

2. Reduction method and reaction conditions

As a reduction method applicable to a method of producing
10 compound (III) by reducing compound (II) in a mixture of compound (I) and compound (II), or a method of producing compound (VI) by reducing compound (V) in a mixture of compound (IV) and compound (V), for example, hydrogenation using a transition metal catalyst, reduction with a hydride
15 reducing agent, a reduction by reaction with an alkali metal or alkaline earth metal, and the like can be mentioned. Of these, hydrogenation using a transition metal catalyst is preferable since reduction of ketone is easily suppressed and only a carbon-carbon double bond is selectively reduced .
20 While the hydrogenation using a transition metal catalyst, which is a preferable embodiment, is explained in the following, the reduction step is not limited to this embodiment.

The hydrogenation is carried out by reacting a mixture of
25 compound (I) and compound (II), or a mixture of compound (IV) and compound (V) with a reducing agent in the presence of a transition metal catalyst.

As the metal of the transition metal catalyst to be used for the hydrogenation, for example, ruthenium, rhodium,
30 iridium, nickel, palladium, platinum and the like can be mentioned. Of these, nickel, palladium and platinum are preferable, and palladium is most preferable. The form of the transition metal catalyst may be a complex catalyst (e.g., tetrakis(triphenylphosphine)palladium, palladium acetate) that

dissolves in a reaction system, or a heterogeneous catalyst (for example, palladium-carbon, palladium hydroxide, palladium black, platinum oxide) that does not dissolve in a reaction system. However, a heterogeneous catalyst is preferable,
5 because it is easily separated from the reaction system, and palladium-carbon and palladium black are particularly preferable.

While the amount of the transition metal catalyst to be used varies depending on the ratio of the compound (III) or
10 compound (V) contained in a raw material mixture, it is generally within the range of 0.01 to 100% by mass, preferably within the range of 0.1 to 10% by mass, relative to the total mass of the raw material mixture.

As the reducing agent, molecular hydrogen, formic acid
15 and a salt thereof and the like can be mentioned, with preference given to molecular hydrogen.

The hydrogen partial pressure when a molecular hydrogen is used as a reducing agent is preferably within the range of 1×10^4 to 1×10^7 Pa, more preferably within the range of 1×10^5 to
20 1×10^6 Pa.

The reaction temperature for the hydrogenation is preferably within the range of 0°C to 150°C, more preferably within the range of 20°C to 100°C. The reaction time varies depending on the reaction conditions, but preferably within
25 the range of 0.1 to 20 hr, more preferably within the range of 1 to 10 hr.

The hydrogenation reaction is generally carried out in the presence of a solvent. The solvent is not particularly limited as long as it does not adversely influence the
30 reaction and, for example, ethers such as tetrahydrofuran, diethyl ether, diisopropyl ether, methyl tert-butyl ether, cyclopropyl methyl ether, dimethoxyethane, 1,4-dioxane and the like; saturated aliphatic hydrocarbons such as pentane, hexane, heptane, octane and the like; aromatic hydrocarbons

such as benzene, toluene, xylene, mesitylene and the like; esters such as methyl acetate, ethyl acetate, butyl acetate, methyl benzoate and the like; alcohols such as methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 1-octanol and the like; nitriles such as acetonitrile and the like; amides such as N,N'-dimethylformamide, N-methylpyrrolidone and the like; dimethyl sulfoxide and the like can be mentioned, which may be used alone or in combination. Of these, ethers such as tetrahydrofuran, diethyl ether, diisopropyl ether, methyl tert-butyl ether, cyclopropyl methyl ether, dimethoxyethane, 1,4-dioxane and the like are preferable, and tetrahydrofuran is more preferable.

While the amount of the solvent to be used is not particularly limited, it is preferably within the range of 1- to 200-fold by mass, more preferably within the range of 3- to 50-fold by mass, relative to the total mass of the raw material mixture.

The method of isolation and purification of compound (III) or compound (VI) obtained by the reduction reaction is not particularly limited, and methods generally used for the isolation and purification of organic compounds can be employed. For example, compound (III) or compound (VI) can be isolated and purified by recrystallization or column chromatography after catalyst removal, extraction operation and the like.

In the reduction reaction, the hydroxyl-protecting group represented by R^1 or R^2 in a mixture of compound (I) and compound (II) may be the same as or different from the hydroxyl-protecting group represented by R^{11} or R^{12} in compound (III). Similarly, the hydroxyl-protecting group represented by R^{21} or R^{22} in a mixture of compound (IV) and compound (V) may be the same as or different from the hydroxyl-protecting group represented by R^{31} or R^{32} in compound (VI). In other words, the hydroxyl-protecting group represented by R^1 , R^2 , R^{21} or R^{22} may

optionally vary by carrying out a reduction reaction as long as it can be deprotected. For example, a chrotonoyl group may change to a butanoyl group as a result of a reduction reaction.

5 In addition, the hydroxyl-protecting group represented by R^1 , R^2 , R^{21} or R^{22} may be eliminated by carrying out the reduction reaction.

3. Deprotection method of hydroxyl-protecting group and reaction conditions

10 The reaction conditions used for elimination of a hydroxyl-protecting group is not particularly limited, and those generally used depending on the kind of the protecting groups can be selected for use.

For example, in the case of a tri-substituted silyl group
15 for which a hydroxyl-protecting group is preferable, compound (VI) is reacted with an acid or fluoride to allow deprotection. While this embodiment is explained in the following, the deprotection is not limited to the embodiment.

The kind of the acid is not particularly limited and, for
20 example, inorganic acids such as hydrochloric acid, sulfuric acid, hydrofluoric acid, hydrobromic acid and the like; organic acids such as acetic acid, trifluoroacetic acid, p-toluenesulfonic acid, methanesulfonic acid and the like; and the like can be mentioned. As the fluorides, for example,
25 tetrabutylammonium fluoride, potassium fluoride, sodium fluoride and the like can be mentioned.

The amount of the acid to be used is within the range of 0.01- to 10-fold by mol, more preferably within the range of 0.1- to 5-fold by mol, relative to compound (VI).

30 The amount of the fluorides to be used is determined based on the number of the protecting groups contained in compound (VI), which are to be eliminated. Preferably, it is within the range of 1- to 10-fold by mol, more preferably within the range of 1- to 5-fold by mol, relative to one

protecting group.

The deprotection may be carried out in the presence of a solvent. Usable solvents are not particularly limited as long as they do not adversely influence the reaction and, for
5 example, ethers such as tetrahydrofuran, diethyl ether, diisopropyl ether, methyl tert-butyl ether, cyclopentyl methyl ether, dimethoxyethane, 1,4-dioxane and the like; saturated aliphatic hydrocarbons such as pentane, hexane, heptane, octane and the like; and the like can be mentioned. Of these,
10 ethers such as tetrahydrofuran, diethyl ether, diisopropyl ether, methyl tert-butyl ether, dimethoxyethane, 1,4-dioxane and the like are preferable, and tetrahydrofuran is more preferable.

When a solvent is to be used, its amount to be used is
15 not particularly limited, but preferably within the range of 1- to 100-fold by mass, more preferably within the range of 3- to 50-fold by mass, relative to compound (VI).

The reaction temperature is preferably within the range of -20°C to 120°C, more preferably within the range of 0°C to
20 80°C. The reaction time is preferably within the range of 0.1 to 20 hr, more preferably within the range of 1 to 10 hr.

The method of isolation and purification of compound (VII) thus obtained is not particularly limited, and methods generally used for the isolation and purification of organic
25 compounds can be employed. For example, compound (VII) can be isolated and purified by recrystallization, column chromatography and the like after extraction operation and the like.

4. Secure supply of raw material

30 The method for producing a mixture of compound (I) and compound (II) and a mixture of compound (IV) and compound (V), which are the raw materials in the present invention, include, for example, reacting (20S)-7 α ,21-dihydroxy-20-methylpregna-1,4-dien-3-one or the derivative wherein a hydroxyl group at

the 21-position is protected(hereinafter to be referred to as 1,4-dien-3-one derivative) with a metal such as alkali metals (e.g., lithium, sodium, potassium etc.), alkaline earth metals (e.g., magnesium, calcium, strontium, barium etc.) and the like. Of these, alkali metals such as lithium, sodium, potassium and the like are preferable, and lithium is more preferable.

The amount of these alkali metal or alkaline earth metal to be used is not particularly limited as long as the reduction of the carbon-carbon double bond at the 4,5-positions of the 1,4-dien-3-one derivative is almost completed and the carbon-carbon double bond at the 1,2-positions can partly remain. However, in order to significantly suppress the reduction of ketone, the amount is generally within the range of 0.8 to 2.5 times the amount necessary for reducing the two carbon-carbon double bonds of the 1,4-dien-3-one derivative. When the amount of alkali metal or alkaline earth metal to be used is less than such range, reduction of the carbon-carbon double bond at the 4,5-positions of the 1,4-dien-3-one derivative tends not to be completed, and when it is greater than such range, side reactions (e.g., reduction of ketone and the like) tend to proceed.

The reaction temperature is preferably within the range of -100°C to 50°C, more preferably within the range of -50°C to 20°C. While the reaction time varies depending on the reaction conditions, it is preferably within the range of 0.1 to 20 hr, more preferably within the range of 1 to 10 hr, from the industrial viewpoints.

The reaction is carried out in the presence of ammonia and/or an amine. The kind of amine is not particularly limited and, for example, linear, branched or cyclic amines having 1 to 6 carbon atoms such as primary amines (e.g., methylamine, ethylamine, isopropylamine, butylamine and the like); secondary amines (e.g., dimethylamine, diethylamine,

diisopropylamine, pyrrolidine, piperidine and the like); polyamines (e.g., ethylenediamine, diaminopropane, N,N'-dimethylethylenediamine and the like); and the like can be mentioned, with preference given to ammonia.

5 The amount of the ammonia and/or amine to be used is preferably within the range of 1- to 100-fold by mass, more preferably within the range of 3- to 50-fold by mass, relative to the 1,4-dien-3-one derivative.

10 In addition, use of a proton donor is necessary for the reaction. While the kind of the proton donor is not particularly limited and, for example, inorganic acids such as hydrochloric acid, sulfuric acid, carbonic acid and the like, carboxylic acids such as formic acid, acetic acid, benzoic acid and the like, ammonium salts or amine salts thereof;
15 water; alcohol and the like can be mentioned, with preference given to alcohol. As the alcohol, for example, linear, branched or cyclic alcohols having 1 to 12 carbon atoms such as primary alcohols (e.g., methanol, ethanol, 1-propanol, 1-butanol, 1-octanol, 1-dodecanol and the like); secondary
20 alcohols (e.g., 2-propanol, 2-butanol, 3-pentanol, cyclopentanol, cyclohexanol, 2-octanol and the like); tertiary alcohols (e.g., tert-butanol, tert-amylalcohol, 2-methylhexanol, 1-methylcyclohexanol and the like); polyhydric alcohols (e.g., ethylene glycol, 1,4-butanediol, 2,4-
25 pentanediol, glycerol and the like); and the like can be mentioned. Of these, tertiary alcohol is preferable, and tert-butanol is more preferable.

30 The amount of the proton donor to be used is generally within the range of 1.5- to 3-fold by mol per one carbon-carbon double bond to be reduced.

 The timing of the addition of the proton donor to the reaction system is not particularly limited and can be freely selected from, for example, a method including addition of a proton donor to the reaction system before reaction of the

1,4-dien-3-one derivative with an alkali metal or alkaline earth metal, a method including addition of a proton donor to the reaction system after reaction of the 1,4-dien-3-one derivative with an alkali metal or alkaline earth metal and
5 the like, with preference given to the former method.

The reaction may be carried out in the presence of a solvent. The usable solvents are not particularly limited as long as they do not adversely influence the reaction and, for example, ethers such as tetrahydrofuran, diethyl ether,
10 diisopropyl ether, methyl tert-butyl ether, cyclopentyl methyl ether, dimethoxyethane, 1,4-dioxane and the like; saturated aliphatic hydrocarbons such as pentane, hexane, heptane, octane and the like; and the like can be mentioned. Of these, ethers such as tetrahydrofuran, diethyl ether, diisopropyl
15 ether, methyl tert-butyl ether, dimethoxyethane, 1,4-dioxane and the like are preferable, and tetrahydrofuran is more preferable.

When a solvent is used, its amount of use is not particularly limited. However, it is preferably within the
20 range of 1- to 100-fold by mass, more preferably within the range of 3- to 50-fold by mass, relative to the 1,4-dien-3-one derivative.

By such reaction, the 1,4-dien-3-one derivative is stereoselectively reduced such that the hydrogen atom at the
25 5-position of pregnane has an α configuration. As used herein, by the stereoselectively is meant that compound (I) and compound (II) are produced in greater amounts than isomers wherein the hydrogen atom at the 5-position of pregnane has a β configuration.

30 The method of isolation and purification of the product after the reaction is not particularly limited, and methods generally used for the isolation and purification of organic compounds can be employed. For example, after elimination of a hydroxyl-protecting group as necessary, a mixture of compound

(I) and compound (II), or compound (IV) and compound (V) can be obtained by concentration after extraction operation and the like.

In addition, (20S)-7 α ,21-dihydroxy-20-methylpregna-1,4-
5 dien-3-one to be the raw material of the 1,4-dien-3-one
derivative can be easily obtained by subjecting 3 α ,7 α -
dihydroxy-5 β -cholanoic acid and/or a salt thereof to a
conversion reaction using a microorganism (JP-B-2525049) to
give 7 α -hydroxy-3-oxo-pregna-1,4-diene-20 α -carbaldehyde, and
10 reducing the 20-position of the compound with sodium
borohydride (WO02/20552), and (20S)-7 α ,21-dihydroxy-20-
methylpregn-4-en-3-one can be easily obtained by subjecting
3 α ,7 α -dihydroxy-5 β -cholanoic acid to a conversion reaction
using a microorganism to give 7 α -hydroxy-3-oxo-pregn-4-ene-
15 20 α -carbaldehyde, and reducing the aldehyde group of the
compound with sodium borohydride (WO03/23047). By protecting
as necessary the hydroxyl groups at the 21-position and the 7-
position of these compounds by a method known *per se*, the raw
material to be used in the present invention can be afforded.

20

Examples

The present invention is explained in more detail in the following by referring to Examples, which are not to be construed as limitative.

Reference Example 1

25 Production of (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-
20-methylpregna-1,4-dien-3-one

Under a nitrogen atmosphere, (20S)-7 α ,21-dihydroxy-20-
methylpregna-1,4-dien-3-one (8.79 g, 25.5 mmol), imidazole
(2.60 g, 38.3 mmol) and tetrahydrofuran (100 ml) were placed
30 in a 200 ml flask, dissolved by stirring and ice-cooled. To
this solution was added dropwise a solution of tert-
butyldimethylchlorosilane (5.00 g, 33.2 mmol) dissolved in
tetrahydrofuran (20 ml) while maintaining the inner
temperature at 0°C to 10°C. After completion of the addition,

the mixture was allowed to warm to room temperature and further stirred for 1 hr. The reaction solution was added to water (200 ml) and the mixture was extracted twice with ethyl acetate (100 ml). The aqueous layer was separated, and the
5 organic layer was washed with saturated brine (100 ml), dried over anhydrous sodium sulfate and concentrated. The obtained crude product was purified by silica gel column chromatography to give (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methylpregna-1,4-dien-3-one (11.11 g, yield 95%) having the
10 following property.

¹H-NMR spectrum (270MHz, CDCl₃, TMS, ppm) δ : 0.03(s, 6H), 0.76(s, 3H), 0.89(s, 9H), 0.99(d, 3H, J=6.9Hz), 1.1-1.8(15H), 2.03(dt, 1H, J=3.0, 12.9Hz), 2.48(dd, 1H, J=3.0, 13.9Hz), 2.75(dt, 1H, J=2.0, 13.9Hz), 3.28(dd, 1H, J=6.9, 9.9Hz),
15 3.56(dd, 1H, J=3.0, 9.9Hz), 4.05(bs, 1H), 6.14(dd, 1H, J=0.9, 2.0Hz), 6.24(dd, 1H, J=2.0, 9.9Hz), 7.08(d, 1H, J=9.9Hz).

Reference Example 2

Production of a mixture of (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-3-one and
20 (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-1-en-3-one

Under a nitrogen atmosphere, tetrahydrofuran (85 ml), (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methylpregna-1,4-dien-3-one (5.00 g, 10.9 mmol) and t-butanol
25 (3.47 g, 46.8 mmol) were placed in a 300 ml three-necked flask. The mixture was cooled to below -50°C and liquid ammonia (85 ml) was added. Then, metal lithium (0.32 g, 46.1 mmol) was slowly added while maintaining at -50°C to -40°C. After completion of the addition, the mixture was further
30 stirred at -40°C for 2 hr. Ammonium acetate (3.61 g, 46.8 mmol) was added to the reaction solution, and the mixture was stirred for 12 hr to remove ammonia while allowing the reaction mixture to warm to room temperature. To the obtained tetrahydrofuran solution was added 15% by mass of an aqueous

sulfuric acid solution to adjust the pH of the aqueous layer to 4 to 6, and the organic layer was separated from the aqueous layer. The organic layer was analyzed by HPLC and found to contain (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-3-one (3.81 g, yield 76%) and (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-1-en-3-one (0.65 g, yield 13%).

HPLC conditions column: TSK-gel80TM, inner diameter 4.6 mm x length 250 mm, column temperature: 35°C, mobile phase: acetonitrile/water=9/1, flow rate: 1 ml/min, detection wavelength: 210 nm, retention time: (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-3-one = 30 min, (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-1-en-3-one = 24 min

Reference Example 3

Production of a mixture of (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one and (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-1-en-3-one

Under a nitrogen atmosphere, a tetrahydrofuran solution containing (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-3-one (3.81 g, 8.2 mmol) and (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-1-en-3-one (0.65 g, 1.4 mmol), obtained in Reference Example 2, was placed in a 200 ml three-necked flask and 6N hydrochloric acid (2 ml) was added, and the mixture was stirred at 40°C for 2 hr. After confirmation of the disappearance of the raw material by TLC, the reaction solution was allowed to cool to room temperature. The aqueous layer was adjusted to pH 8 with 10% by mass of an aqueous sodium hydroxide solution, and the organic layer was separated. The organic layer was analyzed by HPLC and found to contain (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one (2.76 g, yield 96%) and (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-1-en-3-one (0.46 g, yield 95%).

HPLC conditions column: Vydac218TP54 300A5u, inner diameter

4.6 mm x length 250 mm, column temperature: 35°C, mobile phase: acetonitrile/water=3/7, flow rate: 1 ml/min, detection wavelength: 210 nm, retention time: (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one = 30 min, (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-1-en-3-one = 24 min

Example 1

Production of (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one

Under a nitrogen atmosphere, a tetrahydrofuran solution containing (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one (2.76 g, 7.9 mmol) and (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-1-en-3-one (0.46 g, 1.3 mmol), obtained in Reference Example 3, was placed in a 200 ml three-necked flask, 10% palladium-carbon (50 mg) was added thereto and, after substitution with a hydrogen atmosphere, the mixture was reacted at 50°C under an atmospheric pressure for 5 hr. After confirmation of the disappearance of (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-1-en-3-one by HPLC analysis, the reaction mixture was allowed to cool to room temperature. The catalyst was filtered off and the filtrate was concentrated. The obtained crude product was purified by silica gel column chromatography to give (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one (3.06 g) having the following properties {yield 95% (based on the total of (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one contained and (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-1-en-3-one)}.

¹H-NMR spectrum (270MHz, CDCl₃, TMS, ppm) δ : 0.71(s, 3H), 1.01(s, 3H), 1.04(d, 3H, J=6.9Hz), 1.0-2.5(22H), 3.34(dd, 1H, J=6.9, 10.9Hz), 3.61(dd, 1H, J=3.0, 10.9Hz), 3.84-3.85(brs, 1H).

Example 2

Production of (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-3-one

Under a nitrogen atmosphere, a tetrahydrofuran solution containing (20S)-21-tert-butyldimethylsilyloxy-7 α -hydroxy-20-

methyl-5 α -pregn-3-one (4.09 g, 8.8 mmol) and (20S)-21-tert-butyltrimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-1-en-3-one (0.52 g, 1.1 mmol), obtained by the operation similar to that in Reference Example 2, was placed in a 200 ml three-necked flask, 10% palladium-carbon (50 mg) was added thereto and, after substitution with a hydrogen atmosphere, the mixture was reacted at 50°C under an atmospheric pressure for 5 hr. After confirmation of the disappearance of (20S)-21-tert-butyltrimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-1-en-3-one by HPLC analysis, the reaction mixture was allowed to cool to room temperature and the catalyst was filtered off. The filtrate was analyzed by HPLC and found to contain (20S)-21-tert-butyltrimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-3-one (4.47 g) {yield 97% (based on total of (20S)-21-tert-butyltrimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-3-one contained and (20S)-21-tert-butyltrimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-1-en-3-one)}.

Example 3

(20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one

Under a nitrogen atmosphere, a tetrahydrofuran solution containing (20S)-21-tert-butyltrimethylsilyloxy-7 α -hydroxy-20-methyl-5 α -pregn-3-one (4.47 g, 9.7 mmol), obtained in Example 2, was placed in a 200 ml three-necked flask and 6N hydrochloric acid (2 ml) was added, and the mixture was stirred at 40°C for 2 hr. After confirmation of the disappearance of the raw material by TLC, the reaction solution was allowed to cool to room temperature. The aqueous layer was adjusted to pH 8 with 10% by mass of an aqueous sodium hydroxide solution, and the organic layer was separated and concentrated. The obtained crude product was purified by silica gel column chromatography to give (20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one (3.15 g, yield 94%).

Industrial Applicability

Compound (III) and compound (VII) ((20S)-7 α ,21-dihydroxy-20-methyl-5 α -pregn-3-one) produced by the present invention can be easily converted to squalamine by the method
5 described in WO01/79255. Therefore, the method of the present invention can be advantageously used for the production of synthetic intermediates for squalamine.

This application is based on patent application No.
10 108443/2004 filed in Japan on March 31, 2004, the contents of which are hereby incorporated by reference.